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Fragments of MS2 RNA as Messengers for Specific Bacteriophage Proteins: Fragments from Fluorouracil-containing Particles

The RNA of the f2 group of coliphages serves as a polygenic messenger in extracts of *Escherichia coli*, directing the synthesis of at least two of the three known phage proteins, coat protein and RNA synthetase (Nathans, Notani, Schwartz & Zinder, 1962; Capecchi, 1966; Eggen, Oeschger & Nathans, 1967). Fragments of phage RNA have also been shown to have specific messenger activity *in vitro* (Shimura, Moses & Nathans, 1967; Spahr & Gesteland, 1968; Lodish, 1968). By use of chemically defined fragments it may be possible to order the genes in the phage RNA molecule and to determine the effect of one part of the RNA on the translation of another part, i.e. the effect of secondary structure. Evidence has recently been provided by this method for the position of certain genes in the RNA molecule and for the possibility of independent translation of these genes (Engelhardt, Webster & Zinder, 1967; Spahr & Gesteland, 1968; Lodish, 1968; Bassel, 1968). In this paper we present our findings on the *in vitro* translation of fragments of MS2 RNA extracted from RNA-deficient particles which result from growth of MS2 in the presence of 5-fluorouracil (Shimura *et al.*, 1967). The results of our experiments indicate that the fluorouracil–RNA fragments represent about two thirds of the 5′-end of the phage RNA molecule and direct the synthesis of phage coat protein but not the RNA synthetase. An abstract of part of this work has already been published (Shimura & Nathans, 1967).

The preparation and some of the properties of fluorouracil–RNA fragments have been described previously (Shimura *et al.*, 1967). These fragments are somewhat heterogeneous and have an average sedimentation rate of 20 s. Since the defective particles from which the RNA is extracted have about 65% of the normal content of RNA, the 20 s pieces are thought to be about two-thirds the length of complete MS2 RNA. Using the findings of DeWachter, Verhassel & Fiers (1968) and of Glitz (1968) that MS2 RNA has 5′-terminal pppGp, we compared the amount of this tetraphosphate in alkaline hydrolysates of [^{32}P]- or [^{3}H]guanine-labeled RNA fragments and whole molecules of MS2 RNA. As shown in Table 1, the fluorouracil fragments contain about 1·5 times as much pppGp per total nucleotide compared with intact RNA molecules. This result, together with the estimated chain length given above, indicates that the fluorouracil fragments consist largely of the 5′-end two thirds of MS2 RNA.

The activity of the fluorouracil–RNA fragments as messenger in cell-free extracts, as reported previously (Shimura *et al.*, 1967), exceeds that of intact RNA. The fragments direct the synthesis of phage coat protein, as shown by fingerprint analysis and Sephadex filtration (Shimura *et al.*, 1967), and by acrylamide gel electrophoresis (Fig. 1). However, as shown in Figure 1, the fragments do not direct the synthesis of phage RNA synthetase (peak S) whereas whole molecules of MS2 RNA (with or without fluorouracil) direct the synthesis of both RNA synthetase and coat protein. That the fragments code for polypeptide other than the coat protein is shown by

TABLE 1

End group analysis

	RNA	Radioactivity (cts/min) in pppNp	Total radioactivity (cts/min)	Nucleotides per end group
Exp. 1, [^{32}P]guanine				
	MS2—27 s	281	306,000	4240
	FU†—20 s	127	82,700	2610
Exp. 2, [^{3}H]guanine				
	FU—27 s	43	57,200	4840
	FU—20 s	64	52,200	3090

[^{32}P]- or [^{3}H]guanine-labeled MS2 RNA, 27 s FU–RNA, or 20 s FU–RNA were precipitated and washed in the presence of carrier *E. coli* tRNA with cold 0·4 M-perchloric acid. The precipitate was hydrolysed at 35°C for 18 hr in 0·4 N-KOH. After neutralization with perchloric acid and removal of potassium perchlorate, the hydrolysate was electrophoresed on Whatman 3 MM paper in 0·05 M-sodium citrate, pH 5·4, at 30 v/cm for 2 hr. Segments of the paper were then counted. In the case of ^{32}P-labeled RNA, the radioactive nucleotides were first localized by radioautography. The identity of the pppGp spot, which moved beyond GTP and inorganic phosphate, was verified by identification of pyrophosphate and a compound with the mobility of pNp as products of venom phosphodiesterase treatment. In experiment 2 the 27 s and 20 s FU–RNA were extracted from complete and RNA-deficient virus particles, respectively, purified from the same culture.

† *Abbreviation used:* FU, *5-fluorouracil.*

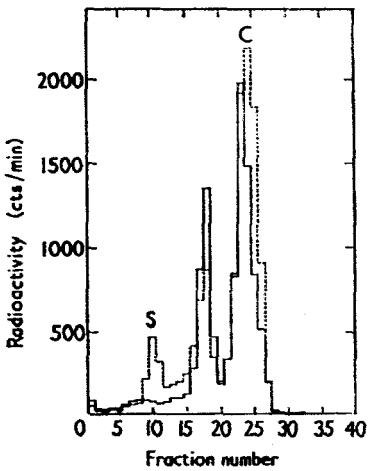

FIG. 1. Gel electrophoresis of cell-free proteins synthesized in the presence of 5-fluorouracil–RNA fragments or intact MS2 RNA.

Protein synthesis was carried out for 40 min at 35°C, as described previously (Nathans, 1965); [^{14}C]threonine was the label with the fragments as messenger (———), and [^{3}H]threonine with intact RNA as messenger (– – – – –). The incubated extracts were mixed, the proteins solubilized with sodium dodecyl sulfate, dialysed and electrophoresed in polyacrylamide gel, as described previously (Nathans, Oeschger, Eggen & Shimura, 1966). Slices of the gel were then counted for ^{14}C and ^{3}H. In separate experiments, intact (27 s) fluorouracil-containing RNA was used as messenger and gave the same results as shown for whole molecules of MS2 RNA. As previously reported (Nathans *et al.*, 1966, and in Eggen & Nathans, 1969), the protein peak labeled S corresponds to the phage RNA synthetase and peak C corresponds to coat protein. The middle peak is peptidyl-tRNA (Schmickel, Eggen & Nathans, manuscript in preparation).

their ability to stimulate the incorporation into protein of histidine, an amino acid missing from the coat protein. Our conclusion from these experiments, which is in agreement with previous reports (Spahr & Gesteland, 1968; Engelhardt *et al.*, 1967) is that the coat protein gene lies within the 5'-end two-thirds of the phage RNA molecule and the RNA synthetase gene is nearer the 3'-(nucleoside) end of the phage RNA than is the gene for the coat protein. At least part of the synthetase gene appears to be in the 3'-end one third of the RNA. Since the third protein coded by the phage RNA, the "maturation protein" or A protein (Lodish, Horiuchi & Zinder, 1965; Heisenberg, 1966; Argetsinger & Gussin, 1966) has not been definitely identified as a cell-free product, the position of its gene relative to the other two is still unknown.

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